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ANGIOGENESIS IN PSORIASIS: THERAPEUTIC IMPLICATIONS*

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ABSTRACT

Within solid neoplasms, the population of tumor cells and the population of capillary endothelial cells constitute a highly integrated ecosystem. Tumor cells release an endothelial mitogen, Tumor-Angiogenesis-Factor (T.A.F.) which continually stimulates new capillaries to grow into the tumor. When T.A.F. is blocked, neovascularization is prevented and tumor nodules stop expanding at a diameter less than 2.5 mm. They enter a dormant phase because they are forced to live by simple diffusion of nutrients and wastes. Thus "anti-angiogenesis" can force a population of tumor cells to become dormant at a tiny diameter.

In this paper an analogy is drawn between tumor angiogenesis and the angiogenesis which accompanies psoriasis. If the relationship between psoriatic epithelium and its capillary endothelium turns out to be similar to the integration of capillaries by solid tumors, then "anti-angiogenesis" may eventually become a useful therapeutic approach in psoriasis.

Commonly, a line of research in one field will facilitate progress in another. If, however, communication between two fields is deficient, progress in each may be retarded because work continues in parallel trenches.

In this paper, I will attempt to draw an analogy between the mechanism of tumor angiogenesis and the angiogenesis which accompanies psoriasis. I have not worked in dermatology, but it is hoped that our studies in tumor angiogenesis will suggest new approaches to understanding the role of angiogenesis in psoriasis.

Neovascularization always accompanies growth of solid malignant tumors. For many years it was thought that vascularization at the edge of an expanding tumor was secondary to waste or necrotic products excreted by the tumor. Tumor vascularization was thought by many to be an "inflammatory" response which held little therapeutic promise.

Recently it has been appreciated that new capillaries are continuously induced by malignant tumors (1, 2, 3). Greenblatt and Shubik (4) and also Ehrmann (5) showed that experimental tumors enclosed in Millipore filters could induce new capillaries on the other side of the filter. We have demonstrated a similar effect by inserting Millipore filters in the subcutaneous air sac of a rat (6).

We have further shown (7) that induction of DNA synthesis in previously resting capillary endothelial cells begins as early as 6 hours from the time a small implant of less than 10^6 tumor cells is injected into the subcutaneous fascia. ^{3}H-thymidine is incorporated in the majority of capillary endothelial cells in a zone extending 3 mm from the edge of this implant. This observation is even more significant against the background of the generally low rate of renewal of capillary endothelial cells in adult animals (8). For example, in the aorta of the adult rat, only 0.1% to 1% of endothelial cells incorporate ^{3}H-thymidine. Trauma may produce a short burst of renewal in which 5% of endothelial cells enter mitosis. But in the neighborhood of a growing tumor the endothelial cell population can achieve a labeling index of 11.4 percent (9) and a doubling time of 50 hours. The rate of renewal is not far behind the doubling time of the malignant tumor cells.

In the present paper, we propose that the neovascularization which accompanies malignant tumors may be analogous in many ways to the neovascularization of psoriasis. By understanding the mechanism of tumor angiogenesis and its therapeutic implications, we may learn a new approach to psoriasis through the pathway of its vascularizing component.

Mechanism of Tumor Angiogenesis

Tumor-angiogenesis-factor (T.A.F.) has been isolated from human and animal tumors in our laboratory (10). T.A.F. is mitogenic to capillary endothelial cells but has no effect on other cells including lymphocytes. Its chemical separation from the cytoplasm of tumor cells has been previously described (11). The factor is an RNA-protein complex of approximately 100,000 molecular weight, destroyed by ribonuclease but not by trypsin. Recent experiments by Tuan (12) in our laboratory have traced it to the nucleus where it appears to be present in the non-histone fraction of chromatin while the histone fraction is entirely negative. T.A.F. has not been found in non-malignant tissue except for placenta. Nor is it

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present in human or mouse leukemia cells. T.A.F. appears not to be species specific in that the fraction separated from human cancer cells causes endothelial mitosis in the rat, mouse, and rabbit. Nor does this factor produce a permanent change in endothelial cells, since withdrawal of T.A.F. is followed by regression of newly formed vessels within a few days.

These experiments suggest that T.A.F. is the mediator of neovascularization in malignant growth. If this is true, it is conceivable that every solid tumor whether it arises from a transformed cell or from a tiny metastatic implant, must go through an early stage living as a small population of cells dependent upon simple diffusion from the extravascular space. Simple diffusion would provide adequate inflow of nutrients and release of catabolites, temporarily. Eventually this tiny colony of cells would reach a diameter (1–2 mm) where simple diffusion would be insufficient. It is just prior to this that new capillaries are stimulated to grow into the tumor. As more cancer cells accumulate so are more capillaries called forth, presumably through the stimulation mediated by T.A.F.

However, if capillaries fail to appear, the tiny unvascularized nodule cannot expand further and becomes sidetracked into a state of permanent dormancy.

Several pieces of evidence support this hypothesis. Folkman *et al.* (13) implanted tumors into isolated perfused organs. These tumors grew rapidly up to 1–2 mm in diameter and then arrested their growth. They never became vascularized because of specific artifacts in the isolated organ which prevented it from generating new capillaries regardless of the stimulus, including wounding (14). The tumor nodule was still viable, however, and when reimplanted into its host animal quickly became vascularized and grew to a size fatal to the animal. These experiments gave us the first insight that blockade of angiogenesis produced a dormant state in a packed population of tumor cells growing in three dimensions. By this blockade of angiogenesis, we did not mean vasoconstriction of old vessels but prevention of the formation of new ones by inhibition of endothelial cell renewal. Greene (15), while carrying out immunological studies of tumors transplanted to the guinea pig eye, observed in a few rare occasions that tumors implanted in the anterior chamber failed to vascularize if they were too distant from the iris. They remained viable but unvascularized for 2 years and never grew beyond a tiny diameter of 1–2 mm.

Recently Gimbrone and Leapman *et al.* (16) in our laboratory have shown that implants of Brown-Pearce carcinoma suspended in the anterior chamber of the rabbit eye, and surviving entirely by simple diffusion, reached a size no larger than 1 mm in diameter and then became dormant. There was no further expansion despite the fact that the tumor implant was viable month after month. Cells in its outer shell would incorporate ^{3}H-thymidine at any time. Histologic sections of such a dormant spheroid showed a thin outer layer of viable and mitosing cells surrounding a larger core of necrotic cells. Cells entering the growth fraction apparently were balanced by dying cells. The dormant spheroid could be transplanted from one eye to another but would not grow as long as it remained suspended in the anterior chamber. However, once the implant was placed upon the iris, new capillaries appeared within 2 days and the tiny mass rapidly increased its volume by 16,000 times its original size over the next 8 days, completely filling the eye.

That such a tumor can be forced into the dormant phase by preventing its neovascularization, is an example of "anti-angiogenesis".

"Anti-angiogenesis"

We have proposed the term "anti-angiogenesis" (17, 18) to introduce a concept of tumor therapy by which tiny neoplastic nodules would remain dormant because their ability to stimulate new capillaries would be blocked. In the rabbit eye experiments, "anti-angiogenesis" was of a mechanical nature. A tumor implant was placed far enough from a vascular bed (the iris), so that outgrowth of new vessels through the aqueous was not possible. A more direct method of "anti-angiogenesis" would be to immunize against T.A.F. so that tumors in proximity to a capillary bed would still be unable to induce neovascularization. The resulting dormant state would leave a tumor at a tiny, barely visible diameter, and perhaps far more vulnerable to chemotherapy than larger tumor masses. Such a tiny population of cells may also be very susceptible to immunotherapy. Whether the frequency of metastases from an unvascularized tumor will be diminished remains to be seen.

We have some evidence from clinical experiments that by nature dormant tumors fail to express their malignant potential and rarely cause symptoms. The best example is the papillary adenocarcinoma of the thyroid which is often found metastasized to the lungs in children and adolescents. Hundreds of tiny nodules may be present in the lungs which upon biopsy are always carcinoma, yet they can remain for longer than 10 years in a stable state during which time the patient is entirely asymptomatic (19).

Mechanism of Dormancy Resulting from "Anti-angiogenesis"

In the previous literature, tumor dormancy has referred to single tumor cells, which have entered a resting phase of the cell cycle. Our definition means a population of cells *in vivo* which has stopped expanding because cell renewal and cell loss are in equilibrium.

Experiments now in progress in our laboratory

may elucidate the mechanism of this dormancy. When tumor cells grow in spheroidal configuration suspended in soft agar, the diameter of the spheroid eventually reaches a critical size of 2–3.5 mm beyond which there is no further expansion (20). Constant replenishment of the medium or isolation of the colony in a large volume of medium will not allow further expansion. Histologic sections show an outer zone of healthy cells which incorporate ^{3}H-thymidine, a middle zone of viable cells which do not incorporate ^{3}H-thymidine, and an inner zone of necrotic cells. At the critical diameter of arrest of further expansion, the number of cells in the growth fraction appears to be just adequate to replace dying cells. Our experiments suggest that at this diameter, the surface area of such a three dimensional spheroid is insufficient to allow the rapid diffusion of catabolites out of the cell population. As the colony enlarges toward the critical diameter, there is a gradual reduction in growth fraction. Accumulation of metabolites may be responsible for this.

The layers of live cells in the outer zone reach a thickness determined by an inward diffusion gradient of oxygen and this layer of live cells never exceeds 200 microns. The diameter of the colony, however, appears to be restricted by catabolite excess due to the peculiar volume-surface relationship of spheroidal configurations where $V \sim S^{3/2}$. Thus, cell populations growing in three dimensions demonstrate self-regulation of growth in the face of unlimited new medium and space because catabolites are accumulated internally. This differs from cell populations growing in two dimensions (flat tissue culture). These systems are *not* self-regulatory and will expand indefinitely in the face of unlimited medium and space because catabolites cannot be accumulated internally.

The histologic sections and the behavior of implants in the anterior chamber were almost identical to those of spheroids in vitro. Thus, we have assumed that a similar mechanism operates in vivo and that the only escape from the dormant situation is the induction of new capillaries. Neovascularization allows the spheroidal population of cells to switch from diffusion to perfusion. This is the reason that exponential growth begins again.

"Anti-angiogenesis" in Psoriasis

We can only speculate that "anti-angiogenesis" might become a useful mode of therapy in psoriasis. The information needed to go further might be obtained by carrying out the following experiments:

1) Is the vascular component of psoriasis true neovascularization?

   Histopathologic sections suggest this but a definitive study is needed to prove that endothelium is rapidly regenerating. A grading system for angiogenesis as developed by Brem in our laboratory might be helpful (21).

2) If psoriasis is accompanied by true neovascularization, are the new capillaries induced by psoriatic epithelium?

   The model for this experiment could be the demonstration by Ryan (22) in which neonatal epidermis induced new capillaries across a Millipore filter in a hamster cheek pouch. Will epithelium from an adult hamster do this or does the property reside only in neonatal or fetal tissue?

3) If neovascularization can be induced by psoriatic epithelium, can a humoral factor responsible for this be separated from psoriatic epithelium? Does the epithelium in psoriasis secrete anything analogous to T.A.F.?

4) Assuming that such a factor could be isolated and that it is the mediator of neovascularization, if it were inhibited for example by immunization against it, would the psoriatic lesions regress or atrophy?

Epithelial-Capillary Relationship

We have previously suggested that a population of tumor cells and the population of capillary endothelial cells which it incorporates may constitute a highly integrated ecosystem. In this relationship the mitotic index of the two cell populations may depend upon each other. Tumor cells appear to stimulate endothelial cell proliferation and endothelial cells may have an indirect effect in controlling the rate of tumor growth.

Psoriatic epithelium may relate in a similar way to its own capillary endothelium. If this analogy is extended, the epithelial-endothelial relationship in psoriasis provides a new insight into the general relationship of renewal systems to the capillaries which supply them.

For convenience, we can divide the renewal systems in the body to high-speed and low-speed. Cells in the intestine, bone marrow, skin, etc. have a rapid doubling time and would be classed as high-speed renewal systems. Cells in the liver, kidney, lung, etc. have a low doubling rate and would be classified as low-speed. It is interesting that under normal conditions, high-speed renewal systems have a mechanism for discharging their cellular products into an open compartment so that they are not inhibited by the accumulation of dying cells. Thus, mucosal cells are discharged into the lumen of the GI tract, bone marrow cells into the bloodstream, endometrium into the vagina, and skin epithelium directly into the outside world. Therefore, in terms of feed-back, high-speed renewal systems under normal conditions are open-looped systems.

Low-speed renewal systems under normal conditions are closed-looped in that the products of their cell division are retained and may act to slow down further cell division. Thus, cells which divide within the liver, the kidney, the lung, etc. have no where to go.

Malignant tumors and psoriasis may be examples of a high speed renewal system which is

"closed" in the sense that the products of cell division cannot be released rapidly enough! In this situation neovascularization is essential to provide run-off for all of the by-products of rapid renewal.

If this model fits with reality, then we might have an explanation of why malignant tumors in the anterior chamber of the eye, are forced into a dormant state when they cannot induce new blood vessels. The dormant state is entered when a previously high speed renewal system cannot get rid of the products of its cell division and these products become inhibitory. A similar situation might hold for psoriatic epithelium. Long before we understand what it is that makes this epithelium regenerate at such an abnormally high rate, we may be able to stop the renewal by stopping neovascularization. For the same reason, "anti-angiogenesis" may in a few years be used to cause dormancy in malignant tumors long before we understand exactly how a neoplastic cell might have been transformed from a normal cell.

REFERENCES

1. Algire, G. H. and Chalkley, H. W.: Vascular reactions of normal and malignant tissues in vivo. I. Vascular reactions of mice to wounds and to normal and neoplastic transplants. J. Nat. Cancer Inst., *6:* 73, 1945.
2. Feigin, I., Allen, L. B., Lipkin, L. and Gross, S. W.: The endothelial hyperplasia of the cerebral blood vessels with brain tumors, and its sarcomatous transformation. Cancer, *11:* 264, 1958.
3. Ide, A. G., Baker, N. H. and Warren, S. L.: Vascularization of the Brown-Pearce rabbit epithelioma transplant as seen in the transparent ear chamber. Amer. J. Roentgenol., *42:* 891, 1939.
4. Greenblatt, M. and Shubik, P.: Tumor angiogenesis: transfilter diffusion studies in the hamster by the transparent chamber technique. J. Nat. Cancer Inst., *41:* 111, 1968.
5. Ehrmann, R. L. and Knoth, M.: Choriocarcinoma: transfilter stimulation of vasoproliferation in the hamster cheek pouch—studied by light and electron microscopy. J. Nat. Cancer Inst., *41:* 1329, 1968.
6. Folkman, J. and Donahoe, P.: Unpublished data.
7. Cavallo, T., Cotran, R., Folkman, J. and Sade, R.: Tumor angiogenesis: rapid induction of endothelial regeneration demonstrated by autoradiography and electron microscopy. J. Cell Biol. In press.
8. Payling-Wright, H.: Endothelial turnover. Thromb. Diath. Haemorrh. (Suppl.), *40:* 79, 1970.
9. Tannock, I. F.: Population kinetics of carcinoma cells, capillary endothelial cells, and fibroblasts in a transplanted mouse mammary tumor. Cancer Res., *30:* 2470, 1970.
10. Folkman, J., Merler, E., Abernathy, C. and Williams, G.: Isolation of a tumor factor responsible for neovascularization. J. Clin. Invest., *49:* 30a, 1970.
11. Folkman, J., Merler, E., Abernathy, C. and Williams, G.: Isolation of a tumor factor responsible for angiogenesis. J. Exp. Med., *133:* 275, 1971.
12. Tuan, D. and Folkman, J.: Unpublished data.
13. Folkman, J. and Gimbrone, M. A., Jr.: Perfusion of the thyroid, *Karolinska Symposia on Research Methods in Reproductive Endocrinology. Fourth Symposium: Perfusion Techniques.* Ed., Diczfalusy, E. Karolinska Institutet, Stockholm, 1971.
14. Gimbrone, M. A., Jr., Aster, R. H., Cotran, R. S., Corkery, J., Jandl, J. H. and Folkman, J.: Preservation of vascular integrity in organs perfused *in vitro* with a platelet-rich medium. Nature, *222:* 33, 1969.
15. Greene, H. S. N.: Heterologous transplantation of mammalian tumors. I. The transfer of rabbit tumors to alien species. J. Exp. Med., *73:* 461, 1941.
16. Gimbrone, M. A., Jr., Leapman, S., Cotran, R. S. and Folkman, J.: Tumor dormancy *in vivo* by prevention of neovascularization. J. Exp. Med. In press.
17. Folkman, J.: Tumor angiogenesis: Therapeutic implications. New Eng. J. Med., *285:* 1182, 1971.
18. Folkman, J.: "Anti-angiogenesis": New concept for therapy of solid tumors. Ann. Surg., *175:* 409, 1972.
19. Winship, T. and Rosvoll, R. V.: Cancer of the thyroid in children. Proc. Nat. Cancer Conf., *6:* 677,
20. Folkman, J. and Hochberg, M.: Self-regulation of three dimensional growth. Unpublished data.
21. Brem, S., Cotran, R. and Folkman, J.: Tumor angiogenesis: a quantitative method for histologic grading. J. Nat. Cancer Inst., *48:* 347, 1972.
22. Nishioka, K. and Ryan, T. J.: Influence of epidermis and other tissues on blood vessel growth in hamster cheek pouch. J. Invest. Derm., *58:* 33, 1972.